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Trichloroacetic Acid Effects on Rat Liver Peroxisomes and Enzyme-Altered Foci

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The initiating and promoting effects of trichloroacetic acid (TCA) were investigated using a rat hepatic enzyme-altered foci bioassay. The experimental protocol used has been shown to induce γ-glutamyltrans-peptidase (GGT)-positive foci in hepatic tissue following an initiating dose with a genotoxic carcinogen. Twenty-four hours following ⅔ partial hepatectomy, rats received either a single oral dose (1500 mg/kg) or 5000 ppm TCA in drinking water for 10, 20, or 30 days. Two weeks after the end of TCA exposure, the rats were promoted for 3 or 6 months with 500 ppm phenobarbital in drinking water. TCA failed to induce GGT-positive foci using this initiation protocol.

In addition, groups of ⅔ partially hepatectomized rats were initiated with a single oral dose of diethyl-nitrosamine (10 mg/kg) and then administered 50, 500, or 5000 ppm TCA drinking water. In this promotion protocol, TCA exposure resulted in a significant increase in the number of GGT-positive foci.

The ability of TCA to stimulate peroxisomal-dependent palmitoyl-coenzyme A oxidation was also investigated. Only the 5000 ppm TCA treatment within the promotion protocol resulted in a significant, although minor, stimulation of peroxisomal enzyme activity.

The findings support the hypothesis that TCA may possess weak promoting activity in the rat liver.

Introduction

The presence of trichloroacetic acid and other nonvolatile halogenated organic products of water chlorination in drinking water has only been recently recognized (*1–6*). Consequently, very few data are available concerning expected environmental levels or what, if any, adverse effects these chemical products may have on biological systems.

Trichloroacetic acid (TCA), dichloroacetic acid (DCA), and chloral hydrate are major nonvolatile chlorinated products formed during chlorination of water containing organic material (*1–5*). What few data are available concerning levels of these compounds in finished drinking water indicate that their consistent presence ranges from ten to several hundred parts per billion (*2,3*). The environmental levels of these nonvolatile chlorination products will certainly vary with local conditions and are directly related to the concentration of humic materials present in the water (*3,5*). Enteric production of TCA and DCA following oral administration of sodium hypochlorite has also been demonstrated (*7*). Although TCA and DCA are structurally similar, chlorination studies of fulvic and humic acids indicate that TCA formation does not proceed through a DCA intermediate, but that both form independently (*3*). The relative concentration of each depends on the reaction conditions (*3,6*).

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Purified TCA and DCA are nonmutagenic in the Ames assay (*8–10*), although some of the nonvolatile by-products formed during water chlorination do exhibit mutagenic activity in the Ames assay (*2*).

TCA is also used as a pre-emergence herbicide, medically as a caustic agent for chemical cautery, and as a common laboratory reagent. These direct uses of TCA are not, however, considered major sources of environmental contamination and exposure.

DCA has direct uses in agriculture as a fungicide and is similarly classified toxicologically as a corrosive. However, during the last decade, DCA has been extensively investigated for potential therapeutic use as a hypoglycemic, hypolactatemic, and hypolipodemic agent (*10–14*). It has been used to treat diabetes mellitus, lactic acidosis, and hypercholestrolemia in man, but because of its toxicity at therapeutic doses, clinical trials have been halted (*10,14*). Although DCA exerts various metabolic effects on many tissues, its hepatic effects are the most prominent (*11,12*). DCA has also been shown to be a metabolite of various hepatotoxic organochlorines such as dichloroethylene, dichloroethane (a hepatic carcinogen in rats), and tetrachloroethane (a hepatic carcinogen in mice) (*15–18*). These halogenated organics are commonly found as pollutants in surface water and groundwater supplies (*19–22*).

TCA is metabolically related to trichloroethylene (TCE), an organic solvent with wide industrial application and a contaminant of surface water and groundwater (*19,22*). TCA, and trichloroethanol are the major animal and human metabolites of TCE (*23–26*). Initially, TCE is oxidized to chloral hydrate, which is either reduced to trichloroethanol or oxidized to TCA. Biotransformation of chloral hydrate results in the formation of the same metabolites. Lifelong, high-dose, oral exposure to TCE has produced hepatocellular carcinomas in mice, but not in rats (*27,28*). More recently, oral administration of TCE has been shown to stimulate hepatic peroxisomal proliferation in mice, but not in rats. However, oral administration of TCA induces hepatic peroxisomal proliferation in both species (*29,30*).

Compounds that are known to induce hepatic peroxisomal proliferation in rodents are a chemically diverse group that includes hypolipodemic agents and industrial plasticizers. Several of these peroxisomal proliferators have been reported to produce liver tumors in rats and mice (*31–33*). These same compounds fail to exhibit mutagenicity in the Ames assay (*31,34*). Recent kinetic studies in TCE-exposed rats and mice indicate that the increased blood levels of TCA in mice compared to rats may explain the greater susceptibility of mice to TCE-elicited hepatocellular carcinomas (*24,25,30*).

The nearly universal practice of drinking water chlorination ensures widespread, low-level, chronic environmental exposure to TCA and DCA. When combined with enteric formation from chlorine ingestion and possible halogenated organic water contamination, the likelihood of significant human exposure and possible toxic effects is increased.

The numerous hepatic effects of TCA and DCA suggest the liver as a probable target organ. Further, the metabolic relationship of TCA to TCE, a hepatic carcinogen, and of DCA to other chlorinated organic hepatic toxicants and carcinogens, makes the assessment of hepatic carcinogenic potential of both compounds a rational objective.

The object of this study was to evaluate the initiating and promoting activity of TCA using a rat liver foci bioassay. The putative preneoplastic enzyme-altered foci were identified by histochemical staining for γ-glutamyltranspeptidase (GGT) activity. Possible hepatic peroxisomal stimulating effects were also investigated.

Materials

Male Sprague-Dawley rats (Washington State University, Pullman WA), 5 to 6 weeks old, were housed in groups of four in stainless-steel, wire-bottomed cages under controlled conditions of temperature, humidity, and lighting. These were given free access to Laboratory Chow (Ralston Purina Co., St. Louis, MO) and deionized drinking water.

Diethylnitrosamine (DEN), reagent grade, was purchased from Sigma Chemical Co. (St. Louis, MO); sodium phenobarbital (PB) and sodium hydroxide, reagent grade, were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ); [1-^{14}C]palmitoyl-CoA was purchased from New England Nuclear Products (Boston, MA); *N-L*-glutamyl-4-methoxy-2-naphthylamine was purchased from Polysciences (Warrington, PA); and TCA, 99+% reagent grade, was purchased from Aldrich Chemical Co. (Milwaukee, WI). Other reagents were purchased from Sigma Chemical Co. or VWR Scientific (Seattle, WA).

Methods

Figure 1 and Table 1 illustrate the design and protocol of the initiation study. Briefly, animals underwent ⅔ partial hepatectomy (*35*) or sham operation (group G) followed 24 hr later by single-dose, oral gavage with DEN, 10 mg/kg (group A), or TCA, 1500 mg/kg (group B). The remaining groups were administered TCA at 5000 ppm in their drinking water for 10, 20, or 30 days. Two weeks following the initiation period, all groups were administered PB (500 ppm) in drinking water for the remainder of the study. Animals were randomly sampled 24 hr after the end of initiation, 24 hr before the start of promotion, and at the 3- and 6-month intervals of the promotion period.

Figure 2 and Table 2 illustrate the design and protocol of the promotion study. Similarly, animals were subjected to partial hepatectomy (PH) or sham operation (group Q), followed 24 hr later by oral gavage with either DEN at 10 mg/kg body weight or distilled water (groups Q and R). Two weeks later, 500 ppm PB or TCA at 50, 500, or 5000 ppm were added to the drinking water for all groups except Group R. Animals were randomly sampled at 2-week, 1-month, 3-month, and 6-month intervals.

To prepare the TCA-containing drinking water, enough TCA was added to deionized water to make the 5000 ppm concentration. This mixture was then titrated to a pH of 6.5 to 8.0 using reagent-grade sodium hydroxide. Tenfold dilutions of this original mixture were used to obtain the 500 ppm and 50 ppm TCA concentrations.

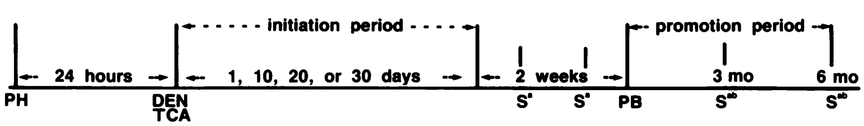

FIGURE 1. Experimental design of enzyme-altered hepatic foci test for initiating activity. PH = partial hepatectomy, DEN = diethylnitrosamine, PB = sodium phenobarbital, and S = sampling interval. a denotes peroxisomal-associated palymitoyl-CoA oxidation assay; b denotes body/organ weights and GGT-positive foci assay.

Table 1. Experimental initiation protocol.[a]

	Group A	Group B	Group C	Group D	Group E	Group F	Group G
PH	+	+	+	+	+	−	+
Initiator	DEN	TCA (gavage)	TCA	TCA	TCA	TCA	—
Days dosed	1	1	10	20	30	30	—
Promotor	PB	PB	PB	PB	PB	PB	PB

[a]DEN was administered in a single oral gavage dose, 10 mg/kg in distilled water; TCA was administered in a single oral gavage dose, 1500 mg/kg in distilled water (noted as TCA in gavage) or was administered in drinking water, 5000 ppm. PB was administered in drinking water 500 ppm.

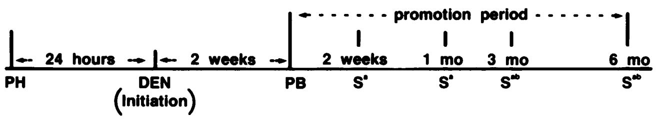

FIGURE 2. Experimental design of enzyme-altered hepatic foci test for promoting activity. PH = partial hepatectomy, DEN = diethylnitrosamine, PB = sodium phenobarbital, and S = sampling interval. a denotes peroxisomal-associated palmitoyl-CoA oxidation assay; b denotes body/organ weights and GGT-positive foci assay.

Quantification of GGT-positive Foci

γ-Glutamyltranspeptidase was detected according to the method of Rutenberg et al. (*36*). Fresh liver sections were collected at the 3- and 6-month sampling periods of both the initiation and promotion protocols, frozen in liquid nitrogen, and stored at −70°C until used.

Sections (5μm) were cut on a cryostat, placed on a slide, air dried, fixed in cold absolute ethanol for 30 min, stained for GGT activity, and counterstained with hematoxylin. GGT-positive foci containing nine or more nuclei were counted directly from the prepared slide using a digitizing pad coupled optically to a microscope. At least 3 cm^2 of liver were assessed from each animal. Foci/cm^2 was evaluated for each animal by dividing the total number of foci present by the total area of liver assessed.

Peroxisomal β-Oxidation Assay

Fresh liver samples were collected at all sampling periods, frozen in liquid nitrogen, and stored at −70°C until used. Liver samples were thawed, weighed, and homogenized in 10 volumes of 0.25 M sucrose using a Brinkmann Polytron Homogenizer (3–15 sec bursts at high speed). The ability of the homogenates to oxidize palmitoyl-coenzyme A (CoA) was measured directly by the oxidation of [1-^{14}C]palmitoyl-CoA according to the method of Lazarow (*37*).

Statistics

All values are expressed as the mean ± standard error of the least squares mean. Statistical significance was determined by analysis of variance and least squares mean multiple comparisons. An α level of $p < 0.05$ was considered to be a significant difference between groups.

Results

Peroxisomal β-Oxidation

TCA induced a significant ($p < 0.05$) and consistent increase in peroxisomal-specific palmitoyl-CoA oxidation only in the high-dose (5000 ppm) groups, P and Q, in the promotion protocol (Table 3). The magnitude of the increase over controls (R and S), 10 to 20%, is small compared to those increases associated with the "typical" peroxisomal proliferators. Clofibrate, fenofibrate, and other known hepatic peroxisomal proliferators induce a 6- to 15-fold increase in peroxisomal-associated palmitoyl-CoA oxidation (*38–40*). A significant ($p < 0.05$) depression of palmitoyl-CoA oxidation was also evident at all sampling periods for the positive control (group M) and appeared to be related to PB treatment.

No significant stimulation of palmitoyl-CoA oxidation was seen at any of the sampling intervals for the TCA-treated initiation groups (Table 4).

Organ and Body Weights

No significant increase in liver weight of TCA-treated rats, compared to controls, was observed at any of the sampling times in either the initiation or promotion groups (Tables 5 and 6). This lack of effect on liver weight is compatible with the minor peroxisomal-associated palmitoyl-CoA stimulating effects produced by high-dose TCA. Hepatomegaly has been shown to accompany the large increases in peroxisomal enzyme activity associated with known hepatic peroxisomal in-

Table 2. Experimental promotion protocol.[a]

	Group M	Group N	Group O	Group P	Group Q	Group R	Group S
PH	+	+	+	+	−	+	+
Initiator	DEN	DEN	DEN	DEN	—	—	DEN
Promotor	PB	TCA (low)	TCA	TCA (high)	TCA (high)	—	—

[a]DEN was administered in a single oral gavage, 10 mg/kg in distilled water; PB was administered in drinking water, 500 ppm; TCA was administered in drinking water, 50 ppm (noted as TCA low), 500 ppm, or 5000 ppm (noted as TCA high).

Table 3. TCA promotion C^{14}-palmitoyl-CoA oxidation.

Group	Treatment	N	Palmitoyl CoA enzyme activity, μm/min/g liver[a]			
			2 weeks	1 month	3 months	6 months
M	PH/DEN/PB	6	0.37 ± 0.01*	0.36 ± 0.02*	0.45 ± 0.04*	0.54 ± 0.02*
N	Ph/DEN/50 ppm TCA	6	0.49 ± 0.02	0.53 ± 0.02	0.59 ± 0.03	0.67 ± 0.02
O	PH/DEN/500 ppm TCA	6	0.55 ± 0.01	0.49 ± 0.01	0.58 ± 0.01	0.64 ± 0.02
P	PH/DEN/5000 ppm TCA	6	0.61 ± 0.01†	0.64 ± 0.01†	0.70 ± 0.02†	0.76 ± 0.02†
Q	5000 ppm TCA	6	0.59 ± 0.02†	0.63 ± 0.01†	0.66 ± 0.02†	0.77 ± 0.02†
R	PH	4	0.52 ± 0.01	0.53 ± 0.01	0.58 ± 0.02	0.66 ± 0.02
S	PH/DEN	4	0.56 ± 0.02	0.51 ± 0.02	0.62 ± 0.01	0.68 ± 0.02

[a] Values are expressed as means ± standard error of the least-squares mean.
*Significantly lower than groups N, O, P, Q, R, and S by least-squares means comparisons ($p \leqslant 0.05$).
†Significantly greater than groups M, N, O, R, and S by least-squares means comparisons ($p \leqslant 0.05$). All other comparisons were not significant.

Table 4. TCA initiation C^{14}-palmitoyl-CoA oxidation.

Group	Treatment	N	Palmitoyl CoA enzyme activity, μm/min/g liver[a]			
			First	Second	3 months	6 months
A	PH/DEN/PB	6	0.49 ± 0.03	0.50 ± 0.02	0.40 ± 0.01	0.57 ± 0.02
B	PH/1 dose/PB	6	0.59 ± 0.04	0.44 ± 0.03	0.42 ± 0.02	0.55 ± 0.02
C	PH/10 days/PB	6	0.54 ± 0.02	0.57 ± 0.08	0.42 ± 0.02	0.58 ± 0.02
D	PH/20 days/PB	6	0.55 ± 0.03	0.48 ± 0.01	0.43 ± 0.03	0.55 ± 0.02
E	PH/30 days/PB	6	0.59 ± 0.03	0.48 ± 0.01	0.37 ± 0.03	0.55 ± 0.02
F	30 days/PB	6	0.57 ± 0.02	0.48 ± 0.01	0.37 ± 0.03	0.59 ± 0.02
G	PH/PB	4	0.55 ± 0.01	0.58 ± 0.05	0.45 ± 0.01	0.57 ± 0.02

[a] Values are expressed as means ± standard error of the least-squares mean. Significant differences are not present between groups by least-squares means comparisons ($p \leqslant 0.05$).

Table 5. TCA initiation/body weights.

Group	Treatment	N	Organ weight as % body weight[a]					
			3 months			6 months		
			Spleen	Liver	Kidney	Spleen	Liver	Kidney
A	PH/DEN/PB	6	0.22 ± 0.15	5.20 ± 0.22	0.43 ± 0.02	0.19 ± 0.01	5.20 ± 0.16	0.43 ± 0.02
B	PH/1 dose/PB	6	0.22 ± 0.15	5.70 ± 0.22	0.44 ± 0.02	0.19 ± 0.01	5.17 ± 0.16	0.40 ± 0.02
C	PH/10 days/PB	6	0.22 ± 0.15	5.96 ± 0.22	0.52 ± 0.02	0.20 ± 0.01	5.04 ± 0.16	0.41 ± 0.02
D	PH/20 days/PB	6	0.26 ± 0.15	5.51 ± 0.22	0.50 ± 0.02	0.20 ± 0.01	4.91 ± 0.16	0.40 ± 0.02
E	PH/30 days/PB	6	0.21 ± 0.15	5.44 ± 0.22	0.49 ± 0.02	0.21 ± 0.01	5.04 ± 0.16	0.43 ± 0.02
F	30 days/PB	6	0.20 ± 0.15	5.44 ± 0.22	0.49 ± 0.02	0.19 ± 0.01	4.97 ± 0.16	0.43 ± 0.02
G	PH/PB	4	0.22 ± 0.18	5.83 ± 0.27	0.44 ± 0.03	0.22 ± 0.01	4.76 ± 0.20	0.45 ± 0.02

[a] Values are expressed as means ± standard error of the least-squares mean. Significant differences are not present between groups by least-squares means comparisons ($p \leqslant 0.05$).

Table 6. TCA promotion organ/body weights.

Group	Treatment	N	Organ weight as % body weight[a]					
			3 months			6 months		
			Spleen	Liver	Kidney	Spleen	Liver	Kidney
M	PH/DEN/PB	6	0.23 ± 0.01	5.47 ± 0.14*	0.53 ± 0.02	0.19 ± 0.13	5.06 ± 0.15*	0.42 ± 0.02
N	PH/DEN/50 ppm TCA	6	0.23 ± 0.01	3.92 ± 0.14	0.52 ± 0.02	0.18 ± 0.13	3.76 ± 0.15	0.40 ± 0.02
O	PH/DEN/500 ppm TCA	6	0.20 ± 0.01	4.12 ± 0.14	0.55 ± 0.02	0.17 ± 0.13	4.41 ± 0.15	0.46 ± 0.02
P	PH/DEN/5000 ppm TCA	6	0.22 ± 0.01	4.25 ± 0.14	0.54 ± 0.02	0.19 ± 0.13	4.41 ± 0.15	0.48 ± 0.02
Q	5000 ppm TCA	6	0.20 ± 0.01	4.19 ± 0.14	0.58 ± 0.02	0.18 ± 0.13	4.44 ± 0.15	0.53 ± 0.02
R	PH	4	0.21 ± 0.02	3.73 ± 0.17	0.53 ± 0.02	0.18 ± 0.15	3.83 ± 0.19	0.46 ± 0.02
S	PH/DEN	4	0.20 ± 0.02	3.90 ± 0.17	0.55 ± 0.02	0.15 ± 0.15	3.96 ± 0.19	0.49 ± 0.02

[a] Values are expressed as means ± standard error of the least-squares mean.
*Significantly greater than groups N, O, P, Q, R, and S by least-squares means comparisons ($p \leqslant 0.05$). All other comparisons were not significant.

Table 7. TCA initiation GGT-positive foci.[a]

Group	Treatment	N	No. of foci/cm² 3 months	No. of foci/cm² 6 months
A	PH/DEN/PB	6	2.05 ± 0.18*	9.93 ± 0.71*
B	PH/1 dose/PB	6	0.05 ± 0.18	0.32 ± 0.71
C	PH/10 days/PB	6	0.08 ± 0.18	0.28 ± 0.71
D	PH/20 days/PB	6	0.07 ± 0.18	0.30 ± 0.71
E	PH/30 days/PB	6	0.06 ± 0.18	0.33 ± 0.71
F	/30 days/PB	6	0.10 ± 0.18	0.49 ± 0.71
G	PH/PB	4	0.07 ± 0.22	0.14 ± 0.86

[a]Values are expressed as means ± standard error of the least-squares mean.

*Significantly greater than groups B, C, D, E, F, and G by least-squares means comparisons ($p \leq 0.05$). All other comparisons were not significant.

Table 8. TCA promotion GGT-positive foci.[a]

Group	Treatment	N	No. of foci/cm² 3 months	No. of foci/cm² 6 months
M	PH/DEN/PB	6	1.65 ± 0.23*	7.61 ± 0.72*
N	PH/DEN/50 ppm TCA	6	0.71 ± 1.16†	1.83 ± 0.32‡
O	PH/DEN/500 ppm TCA	6	0.39 ± 0.16	1.63 ± 0.32‡
P	PH/DEN/5000 ppm TCA	6	0.70 ± 0.16†	2.45 ± 0.32‡
Q	5000 ppm TCA	6	0.23 ± 0.16	0.03 ± 0.32
R	PH	4	0.23 ± 0.20	0.41 ± 0.39
S	PH/DEN	4	0.05 ± 0.20	0.30 ± 0.39

[a]Values are expressed as means ± standard error of the least-squares mean.

*Significantly greater than groups N, O, P, Q, R, and S by least-squares means comparisons ($p \leq 0.05$).

†Significantly greater than groups Q and S by least-squares means comparisons ($p \leq 0.05$). Group M excluded from comparisons.

‡Significantly greater than groups Q, R, and S by least-squares means comparison ($p \leq 0.05$). Group M excluded from comparisons.

ducers (*31*). No differences in body or organ weights could be attributed to TCA administration. Additionally, no necrosis was observed in the liver in any groups treated with TCA.

A significant ($p < 0.05$) increase in liver weight was detected in the positive control (group M) of the promotion groups (Table 6). This increase is consistent with hepatomegaly because of microsomal induction commonly seen with PB treatment.

TCA Initiation

The results of the GGT-positive foci initiation bioassay are summarized in Table 7. Only the positive control (group A), which had approximately 2 and 10 foci/cm² at 3 and 6 months, respectively, showed a statistically significant effect. The initiation control (group G) had almost no induction of GGT-positive foci. These results are consistent with those of other investigators (*41–43*), who have shown that both PH and PB promotion are necessary to optimize the induction of DEN-initiated enzyme-altered foci. The four TCA treatment groups (B, C, D, and E) failed to demonstrate significant induction of GGT-positive foci. The differences in size of foci among the groups have not yet been statistically evaluated.

TCA Promotion

The results of the promotion experiment are summarized in Table 8. As with the initiation protocol results, the positive control (group M) had induced GGT-positive foci at a level significantly ($p < 0.05$) higher than that seen in the other groups at both the 3- and 6-month intervals. The lack of significant foci induction within the promotion controls (group S) or initiation/promotion controls (group R) again supports the need for both PH and PB promotion to optimize induction of DEN-initiated foci. The low-dose (50 ppm) TCA-promotion group (N) had significantly ($p < 0.05$) greater foci induction at 3 months than any of the negative controls (groups Q, R, and S), except for group R. This same level of foci induction is seen with high-dose (5000 ppm) TCA promotion (group P). The statistical differences between the low and high TCA dose groups (N and P) and control group R were $p < 0.06$ and $p < 0.07$, respectively. The level of GGT-positive foci induction seen at 3 months with 500 ppm TCA promotion (group O) was greater than all the negative controls but was not statistically significant. However, at the 6-month interval, all three dose levels of TCA promotion (groups N, O, and P) resulted in statistically significant ($p < 0.05$) greater levels of foci induction compared to any of the negative controls (groups Q, R, and S).

Discussion

It has been recently reported that TCA induces hepatic peroxisomal enzyme activities (*29,30*). This peroxisomal stimulating activity, along with increased metabolic TCA formation in the mouse compared to the rat following TCE administration has led several researchers to speculate that TCA levels may be important for explaining why TCE is carcinogenic in the mouse but not in the rat (*25,26*).

In this study, when TCA was investigated for its initiating potential in the rat hepatic foci bioassay, no evidence of significant genotoxicity was found. Short-term *in vitro* mutagenicity testing of TCA has also been negative (*8,9*). Although there appears to be little to support the notion of significant genotoxic TCA activity, the paucity of data does not allow a definitive determination at this time.

The promoting activity of TCA was also investigated using the rat hepatic system. After 3 months of TCA administration in drinking water, significant, although somewhat equivocal, promotion activity was observed for both the low dose (50 ppm) and high dose (5000 ppm) of TCA. The promoting activity associated with the medium dose of TCA (500 ppm), although resulting in more GGT-positive foci than those of the negative controls, was not significant. However, by 6 months, all three dose levels of TCA produced significant increases in the number of GGT-positive foci. This promoting activity was not, however, of the magnitude seen with phenobarbital, a known potent hepatic tumor promotor. No dose-response relationship between the weak promoting activity and the concentrations of TCA used in this study was evident.

Although TCA is reported to cause hepatic peroxisomal stimulation in rats and mice, the results of this study indicate that it is unlikely that TCA's effects are related to the promoting ability seen here. The minimal stimulation, 10 to 20% over controls, of peroxisomal-associated, cyanide-insensitive, palmitoyl-CoA oxidation in TCA-exposed rats was seen only at the 5000 ppm level and only within the promotion protocol. This finding is in contrast to the promoting activity seen at all three concentrations of TCA. The lack of hepatomegaly associated with TCA administration is further evidence of TCA's weak ability to stimulate hepatic peroxisomes. Known hepatic peroxisomal proliferators have been shown to induce an associated hepatomegaly (*40*).

This study provides evidence that TCA is a possible weak, epigenetic carcinogen. It should be pointed out that no hepatocellular carcinomas or other hepatic tumors were found in any of the experimental animals used in this study. Further research is needed to verify possible carcinogenic effects of TCA in other bioassay systems.

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